Concentrations of Functional Lipids in Abraded Fractions of Hulless Barley and Effect of Storage

K.S. Liu and R.A. Moreau

ABSTRACT: Hulless barley kernels were sequentially abraded to achieve 4%, 8%, 16%, 24%, 32%, and 40% removal. Abraded fines, kernels, and ground kernels were stored at 35 °C and 75% relative humidity for 3 wk. Stored samples were extracted and the levels of oil, free phytosterols, tocopherols (Ts), and tocotrienols (T3s) were analyzed and compared with freshly abraded fractions. The results revealed that oil, sterols, and Ts were concentrated in the outer layers, particularly in the germ layer. In whole kernels, homologues of both Ts and T3s showed the same ranking order in concentrations as $\alpha > \gamma > \beta > \delta$. The homologue composition of Ts remained the same but that of T3s changed across the kernel. The %T3 in total tocols increased in fractions with increasing endosperm tissue. Storage caused no change in oil and Ts but significant changes in sterols and T3s. The changes were differential among T3 isomers, with α-T3 decreasing and δ-T3 increasing. The degradation of α-T3 was accelerated in fractions with more endosperm tissue. Grinding kernel samples before storage accelerated sterol degradation but had a limited effect on changes of T3s. A 2nd experiment using a different hulless barley line and ambient storage for 6 mo confirmed all the findings except that the changing trend for sterols was inconsistent. These results provide practical information to those who wish to produce a barley fraction enriched with a particular functional lipid and maintain stability of their products.

Keywords: hulless barley, pearling, phytosterols, storage, tocols

Introduction

Tocols (vitamin E) are grouped into 2 main classes, tocopherols (Ts) and tocotrienols (T3s), based on their prenyl side chains. T3s have an unsaturated side chain whereas the Ts have a saturated side chain. Each class consists of 4 principal homologues (isomers), termed α, β, γ, and δ, depending on the methylation pattern on the chromanol ring. Although all the isomers are physiologically active in alleviating symptoms of vitamin E deficiency, α-T has the highest vitamin E activity (Barnes 1983). Tocols are also known to promote health through their antioxidant activity and cholesterol-lowering ability (Yang 2003). Phytosterols are also one of the food components currently being actively studied. Their potency in decreasing serum cholesterol levels and thus protecting against cardiovascular diseases (Hallikainen and others 2000) has led to the development of functional foods enriched in phytosterols.

Cereal grains and certain vegetable oils are good sources of tocols, but the concentration and composition (8 possible isomers) of tocols vary considerably among sources (Barnes 1983). Barley contains high levels of both Ts and T3s and is one of the few plant materials that contain significant levels of all 4 T and all 4 T3 isomers (Piironen and others 1986; Yang 2003). Cereal products are also recognized as significant sources of phytosterols. In cereals, phytosterols occur as free sterols, steryl esters with fatty acids or phenolic acids, steryl glycosides, and acylated steryl glycosides (Piironen and others 2002). The levels of these components vary in different cereals and in different milled fractions or parts of kernels (Dutta and Appelqvist 1996; Piironen and others 2002; Moreau and others 2007a).

In previous studies, we quantitatively analyzed the levels of phytosterols and tocols in barley fractions after pearling for 90 s (Lampi and others 2004) or scarification for 60 s (Moreau and others 2007a). The results indicated that the total levels of phytosterols, Ts, and T3s in abraded fractions from barley were probably not high enough to justify the use of barley as a functional food but their levels in the oil extracted from kernels and abraded fines were sufficiently high to consider the potential use of barley oil as a new functional oil. A subsequent study (Moreau and others 2007b) further examined the levels of functional lipids in subfractions with a range of particle size, obtained by sieving abraded fines.

While it is possible to concentrate barley oil and oil-soluble components in certain fractions through processing (Wang and others 1993; Peterson 1994; Moreau and others 2007a, 2007b), there are obvious concerns about their stability and shelf life in barley products (such as abraded fractions), because of possible deterioration during processing and storage. The total tocol concentration in barley flour decreased at the rate of 5% per week when the flour was exposed to light at 25 °C over the 8-wk storage (Tyopponen and Hakkarainen 1985) but in intact barley grain it decreased at about 1% per month during 11 mo of storage in a conventional silo (Hakkarainen and others 1983). Fastnaught and others (2006) studied the changes in free fatty acids and an oxidation coproduct during storage of milled hulless barley products, but analyses of phytosterols and tocols were not included in their study.

In the current study, we sequentially abraded seeds of 2 hulless barley genotypes into several surface layer (fines) fractions and

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corresponding abraded kernels, subjected abraded samples of one genotype to an accelerated storage and samples of another genotype to a natural storage, and examined the yields of oil and oil-soluble components (functional lipids). The objectives of this study were severalfold: (1) to investigate the yield and distribution of oil and functional lipids within a hulless barley kernel, (2) to study the effect of storage on the yield and distribution of these attributes, and (3) to investigate changes of the homologue composition of tocols within the barley kernel and effect of storage on the composition. This information should be useful for those who want to produce a functional lipid product from barley and maintain product stability.

Materials and Methods

Materials

Two hulless barley lines, 00AH3778 and 03HR3052, were gifts from Dr. Don Obert of USDA-ARS at Aberdeen, Idaho, U.S.A. and used as the experimental materials. They were grown in 2006 at 2 different locations (Aberdeen and Soda Spring, respectively), Idaho, and harvested at slightly different time. Seeds were stored in a cold room before the experiment started. The 1st line was used for the experiment with accelerated storage, while the 2nd line was for the confirmatory experiment conducted under natural storage conditions. The intact seed samples were placed in a cold room (about 5 °C) soon after harvest and stored there for about 3 mo. The seeds were passed through a screen to remove broken kernels and any foreign material, before being subjected to treatments. The treatments included (1) abrading seeds into surface layer (fines) fractions and corresponding abraded kernels, (2) storage, (3) grinding kernel fractions into particulates, and (4) various combinations of these treatments.

Abrading kernels into fractions

A general procedure employing a laboratory electrical seed scarifer (Forsberg Inc., Thief River Falls, Minn., U.S.A.) was used to abrade hulless seeds into different fines fractions and corresponding kernel fractions (Liu 2007). The tabletop machine consisted of a metal drum with its inner surface mounted with 40-grit sandpaper and a horizontal rotating steel propeller that is mounted at the center of a metal cylinder. The propeller was driven by a 1/3 hp motor.

Seed samples were subjected to several successive cycles of abrasive milling. The first 2 cycles each removed about 4% of outer layers, while each of the subsequent cycles removed about 8% of outer layers. For each cycle, 20 g of original seed or abraded kernel sample were put into the drum. The drum was horizontally aligned into the cylinder with the propeller fixed at the center. The motor was started and then stopped after an estimated percentage of grain surface (outer layer) was removed. Abraded kernels, mixed with fines, were removed from the chamber and brushed into a container. The mixture was sifted over an 18-mesh (U.S standard, corresponding to 1.00-mm opening) screen with the outer layer fraction passing through the screen. The abraded kernels that remained on the screen were weighed. The percentage of outer layer removal was determined using the following formula: surface removal (%) = [(initial sample weight – abraded kernel weight)/initial sample weight] × 100.

Samples of fines removed at each abrading stage were named FN1, FN2, FN3, and so on. The corresponding abraded kernels were named AK1, AK2, AK3, and so on. For the 1st experiment with accelerated storage, 6 cycles of abrading were made on the original seed sample, with the final abraded fractions having about 40% of total surface layer removal. For the 2nd confirmation study, only 5 cycles of abrading were made, with the final abraded fractions having about 32% surface removal. In both experiments, duplicate samples were prepared for each treatment. For controls, seeds were abraded into fractions immediately before extraction and chemical analyses were conducted.

Storage

For the 1st experiment, the intact kernel fraction and 6 abraded kernel fractions were divided into 2 portions, with 1 portion finely ground into flour. Abraded fines, kernels, and ground kernels were subjected to accelerated storage in an airtight incubator (a vacuum oven, operated at ambient air pressure), with a temperature preset at 35 °C, for 3 wk. The relative humidity (RH) was maintained at about 75% by placing 2 beakers of a saturated NaCl solution inside the oven. Individual samples were packed in paper envelopes and sealed. For the 2nd experiment, abraded fines, corresponding abraded kernels, and the original intact kernels were all stored in sealed plastic bags at ambient temperature (approximately 25 °C) and ambient relative humidity (a temperature-controlled seed laboratory) for 6 mo. For this experiment, ground kernels were not included as a treatment before storage. The initial moisture contents of the seed and fraction samples were in the range of 8% to 10%.

Grinding

All kernels, either before or after storage or freshly abraded, based on the type of treatments, were milled with a Cyclone Sample Mill (UDY Corp., Fort Collins, Colo., U.S.A.) with mill enclosures, a vacuum system, and a sieve with 1.0-mm round openings. Fines (surface layer) were not milled since the particle size was already fine enough to pass through a 1.0-mm sieve.

Chemical analysis

The fines and ground kernels were extracted with hexane and analyzed for the yields of oil, total free sterols, 4 T isomers, and 4 T3 isomers. Oil yield was expressed as percentage of original dry seed mass, sterol yield was expressed as g/100 g extracted oil, and tocol yield was expressed as mg/100 g extracted oil. Total T yield, total T3 yield, total tocol yields (Ts + T3s), and %T3s were also calculated.

Triplicate oil extractions for each sample were performed by weighing 0.5 to 2 g of each scarified and/or ground sample into a 55-mL glass tube with a Teflon cap. Hexane (40 mL) was added to each tube. The slurry was then ground with a Polytron Homogenizer (Brinkman Inc., Westbury, N.Y., U.S.A.) for 3 × 15 s. The tubes containing the ground slurry were shaken horizontally for 1 h in a Burrel Wrist Action Shaker (Burrel Inc., Pittsburgh, Pa., U.S.A.). The extract was filtered through Whatman GF/A glass fiber filters (Thomas Scientific, Swedesboro, N.J., U.S.A.), and dried under a stream of nitrogen. After the mass was recorded the residue was dissolved at a concentration of 1 mg/mL in hexane (with 0.01% BHT) for HPLC analysis.

Tocopherols and tocotrienols were quantified based on a HPLC method described elsewhere (Moreau and others 2007a), using a fluorescence detector. Free phytosterols were measured based on another HPLC method, also described elsewhere (Moreau and others 2007b), using an evaporative light-scattering detector. Moisture was measured by an official method (AOAC 2000). It was used for converting fresh weight basis to dry matter basis for all other attributes measured.

Data treatments and statistical analysis

Data were treated with a JMP software, version 5 (JMP, a business unit of SAS Inst Inc., Cary, N.C., U.S.A.) for calculation of means and standard deviation and for analysis of variance (ANOVA) to

determine the effect of treatments on all measured attributes (except for the moisture level). The significant level was set at $P < 0.05$. The data presented are means ± SD.

Results and Discussion

In the 1st experiment, hulless barley kernels (00AH3778) were sequentially abraded via scarification for 6 cycles to generate 6 "fines" (surface) fractions (FN1-6) that represented 4%, 8%, 16%, 24%, 32%, and 40% of removal of kernel mass. The intact kernel (AK0) fraction plus 6 corresponding "abraded kernel" fractions (AK1-6) represented about 100%, 96%, 92%, 84%, 76%, 68%, and 60%, respectively, of the original whole kernel mass. A germination test conducted in our laboratory showed that, during abrading hulless barleys, germ (embryo) damage or removal started at about 5% of outer layer removal and that complete removal of germs occurred at about 11% removal of surface mass. Based on this observation and microscopic examination, we could reasonably determine the types of major tissues that constituted each fraction obtained after sequential scarification (Table 1).

Oil yield, distribution within barley seeds, and effect of accelerated storage

The oil yield in the intact seed for the hulless line, 00AH3778, was a little over 2% (Figure 1A, 0% surface removal). This value was in agreement with previous reports (Wang and others 1993; Moreau and others 2007a). As the level of surface removal progressed, the oil yield gradually decreased in the remaining kernel. At about 40% removal level, the abraded kernel, representing about 60% of original kernel mass, had less than 1% oil yield. Thus, there is still some measurable oil in the endosperm tissue. The decrease in oil yield was due to the large concentration of oil in the surface layer fractions. For the fines fractions with initial 4 to 8% removal levels, the oil yield was about 5 times greater than that of the corresponding kernel fraction. As the removal level increased to 40%, the oil yield in the fines fractions dramatically decreased to the level of the original whole kernel. Accelerated storage did not cause any apparent changes in the yield of extractable oil. This is expected since storage typically affects quality not quantity of total extractable lipids. Storage of ground samples also did not cause a significant change in the oil content.

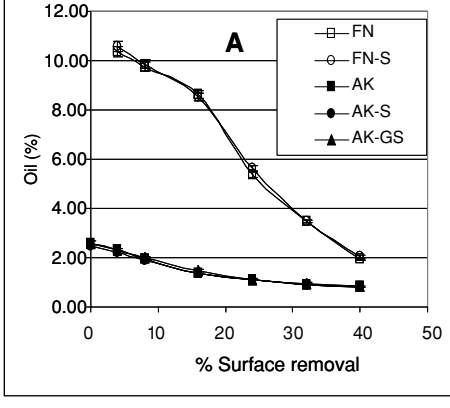
Phytosterol yield and distribution within barley seeds and effect of accelerated storage

Similar to oil, free phytosterols were also concentrated in the surface layers, but the extent was not as large as for oil (Figure 1B). The phytosterol yield in the fines fractions with 4% or 8% removal was about 3 times higher than the phytosterol yield of the corresponding kernel fractions. In the kernel samples without accelerated storage, when surface removal levels reached about 40%, the yield of phytosterols was about 40% of that in the intact kernel. Thus, like total oil, phytosterols remained at a low but significant concentration in the endosperm. Furthermore, both storage and grinding before storage had significant effects on phytosterol yield and its distribution pattern within the kernel. Accelerated storage caused a significant decrease in phytosterols in both types of fractions, fines, and abraded kernels. When kernels were ground before storage, there was a further decrease in phytosterol yield. The decrease was so extensive that its concentration in most kernel

Table 1 — Description of different fractions after abrading hulless barley seeds by the lab method described in Materials and Methods.

Fraction name	Symbol	Type of fraction	Cumulative surface removal (%)[a]	Percentage of original kernel[a]	Major tissues[b]
First layer fines	FN1	Outer layer	4	4	Germ, pericarp, and testa
Second layer fines	FN2	Outer layer	8	8	Germ, pericarp, testa and aleurone
Third layer fines	FN3	Outer layer	16	16	Aleurone
Fourth layer fines	FN4	Outer layer	24	24	Aleurone and subaleurone
Fifth layer fines	FN5	Outer layer	32	32	Subaleurone and endosperm
Sixth layer fines	FN6	Outer layer	40	40	Endosperm
Whole kernel	AK0	Kernel	0	100	All seed tissues
First abraded kernel	AK1	Kernel	4	96	Endosperm, aleurone, and germ
Second abraded kernel	AK2	Kernel	8	92	Endosperm, aleurone, and subaleurone
Third abraded kernel	AK3	Kernel	16	84	Endosperm and subaleurone
Fourth abraded kernel	AK4	Kernel	24	76	Endosperm and subaleurone
Fifth abraded kernel	AK5	Kernel	32	68	Endosperm
Sixth abraded kernel	AK6	Kernel	40	60	Endosperm

[a]Approximate values of average of several runs at each cycle, weight basis.
[b]Major tissues in each fraction were predicted, based on visual observation.



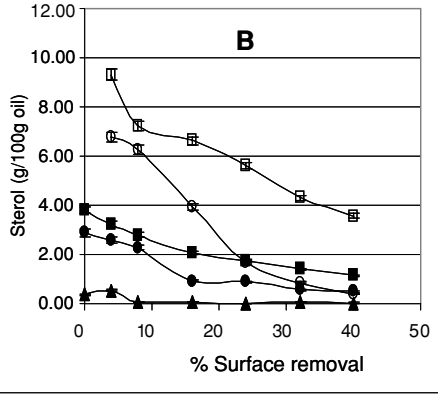


Figure 1 — The yield and distribution of oil (A) and total free sterols (B) in abraded barley fines and kernels and the effect of accelerated storage. FN = fines; FN-S = stored fines; AK = abraded kernels; AK-S = stored abraded kernels; AK-GS = ground and then stored abraded kernels.

fractions that were ground and then stored was near zero. These observations indicate that free sterols were not stable during storage and their degradation was accelerated in samples with more surface area.

Dutta and Appelqvist (1996) reported that the levels of total phytosterols in wheat, oats, barley, and rye were 44.6, 12.1, 35.6, and 71.2 mg/g of extracted lipids, respectively. It should be pointed out here that the oil content varies among these grains too. Our data on whole barley kernels without accelerated storage show a value of 3.8 g/100 g extracted oil, thus agreeing with their reported value. Piironen and others (2002) reported not only the total phytosterol yield (the sum of free and covalently bound sterols) but also the distribution of phytosterols in several cereals (barley, oats, rye, and wheat) and in milled fractions of rye and wheat. They also found that the levels of total phytosterols and the levels of the individual phytosterols varied in different cereals and that they were lower in most refined rye and wheat flours than in the bran fractions.

T yield, distribution within barley seeds, and effect of accelerated storage

As expected, all 4 T homologues were detected in the barley samples (Figure 2). Among them the ranking order for the concentration in the intact barley seed, expressed as mg/100 g hexane extracted oil, was $\alpha > \gamma > \beta > \delta$. With respect to distribution within the kernel, the 4 T isomers showed a similar pattern. They were all highest in the outer layers and decreased gradually to zero at the inner core areas (about 32% surface removal). For the fines fractions, the sharpest decrease was within the 1st 16% removal levels. These observations indicate that Ts were mostly concentrated in germ and aleurone layers but were absent in endosperm. Since all homologues exhibited similar patterns in both types of fractions (bran and kernel), the isomer composition of Ts remained relatively unchanged as the level of surface removal increased. Furthermore, no significant storage effect ($P < 0.05$) was observed in the levels of all T isomers in abraded barley fractions. This was also true for grinding followed by storage. Interestingly, however, for the whole kernel samples (0% removal) there were some detectable differences in all the isomers among treatments of control, storage, and grinding and then storage. Storage of intact whole kernel caused a small increase in the levels of all of the Ts, compared with the control (nonstorage), while storage of ground seeds led to a small decrease in all of the Ts.

T3 yield, distribution within barley seeds, and effect of accelerated storage

All 4 T3 isomers were detected in the barley seeds (Figure 3). The ranking order in isomer concentration in the whole seeds was $\alpha > \gamma > \beta > \delta$, the same as that for T isomers. However, the distribution patterns of concentration for 4 T3 isomers were very different from those of T isomers. The α-, γ-, and δ-T3 isomers exhibited a similar pattern: lowest at the seed coat layer, increasing in the aleurone area, and decreasing slightly but remaining relatively high in the endosperm tissue. However, the extent of the changes due to progressing surface removal levels varied among the T3 isomers, particularly for abraded kernel fractions. For example, γ-T3 in kernel fractions decreased at a much faster rate than α and δ-T3, when removal levels were between 8% and 40%. β-T3 exhibited a different pattern; lowest at the seed surface, and gradually increasing to the highest level toward the inner endosperm area. For all of the T3 isomers, the sharpest increase was in the fines fractions, within the first 16% removal levels. This sharp increase was mainly due to a much lower concentration in the initial fines fraction (4% removal). These observations indicate that unlike Ts, the concentrations of T3s were lowest in the germ and seed coat area, reached the highest levels in the aleurone layer, and remained high in the endosperm tissue. An exception was β-T3, which was present in the highest concentration in the endosperm area.

Because the concentration distribution varied among all T3 homologues, either in the extent of changes or the entire pattern, the homologue composition of T3s changed across the barley seed

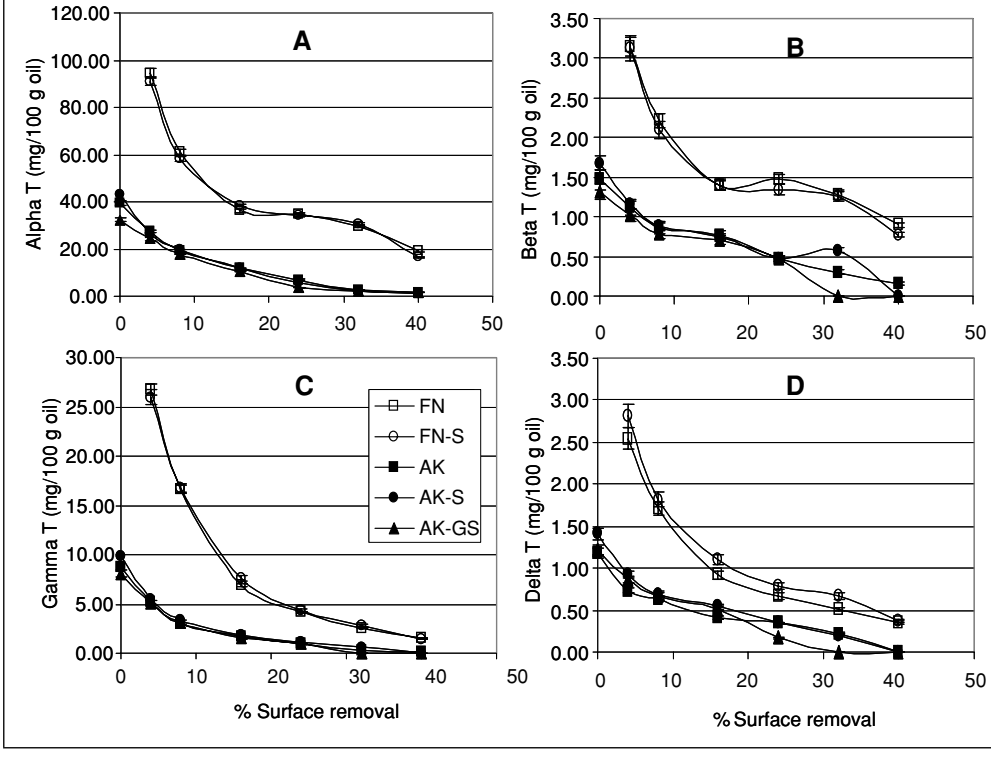


Figure 2—The yield and distribution of α (A), β (B), γ (C), and δ (D) tocopherol isomers in abraded barley fines and kernels, and the effect of accelerated storage. FN = fines; FN-S = stored fines; AK = abraded kernels; AK-S = stored abraded kernels; AK-GS = ground and then stored abraded kernels.

kernel. In particular, at the inner endosperm region, the relative percentage of β-T3 increased while that of γ-T3 decreased compared with those of the whole kernel and the bran region.

Furthermore, storage had a significant effect on the yield and distribution patterns of all T3 isomers. Most interestingly, the direction and the extent of changes resulting from the treatment varied with T3 homologues, type of tissues, and levels of surface removal. α-T3 and δ-T3 were affected most by the treatment. Yet they showed an opposite trend. Storage caused a significant decrease in α-T3 and the extent of this decrease was more pronounced as the surface removal progressed. It was also more pronounced in kernel fractions than in fines fractions. In contrast, δ-T3 increased in the fines fractions as a result of storage and the extent of the change increased with surface removal levels. In abraded kernel fractions, it also increased during storage, but the extent of the change did not vary with surface removal levels. γ-T3 was least affected by storage, but for β-T3, storage caused a decrease only in kernel fractions with surface removal levels higher than 16%. Because storage had a differential effect on T3 homologues, it also caused changes in the relative percentages of each T3 isomer in a particular fraction sample.

The observation that storage caused significant changes in T3s but had no effect on Ts can be attributed to their difference in molecular structure. T3s have an unsaturated side chain whereas the Ts have a saturated side chain. Molecules with unsaturated bonds tend to be more susceptible to oxidation and perhaps other reactions than those with saturated bonds. Furthermore, the observation that storage-induced degradation of α and β-T3 was accelerated in fractions with increasing levels of surface removal can be explained by another observation in the same study that all T homologues are concentrated in the surface layer fractions and absent in endosperm tissue. Apparently, Ts offered a protective effect on T3s during storage.

Unlike for free phytosterols where grinding before storage of kernel fractions caused further change as compared with storage of unground kernel fractions, for T3s grinding kernels before storage exhibited some but limited effect on storage-induced changes. This implies that unlike for sterols, storage-induced changes of T3s do not necessarily accelerate in samples with more surface area.

Total tocols and %T3s

Because Ts and T3s exhibited opposite distribution patterns and because compared with T counterparts, α and β homologues of T3 were present in higher concentrations, the yield of total tocols (Ts + T3s) exhibited a balanced pattern across the seed kernel (Figure 4C): relatively high in the outermost layers, increased to the highest level at the aleurone, and slightly decreased in the endosperm region. If we had measured only the total tocol yield, the contrasting change in patterns between Ts and T3s within the kernel would probably not have been evident. As noted earlier, storage had no noticeable effect on Ts but it did affect T3s. Yet, because T3s occurred in higher concentration than Ts, particularly for the α isomer, the largest homologue within both T and T3 groups, storage exhibited a significant effect on total tocols, particularly for abraded kernels.

Interestingly, when we examined the %T3 in the total tocols, the value was the lowest in the outer surface layers, increased sharply in the aleurone region, and then slightly increased toward the inner core area. The %T3 increased from about 40% to 92% and from 78% to 99% in the fines and abraded kernels fractions, respectively. However, the %T3 was not affected by storage in any of the fractions. This can partially be explained by their opposite distribution patterns within the barley kernel and a possible protective effect of Ts on storage-induced changes of T3s.

Distribution of individual functional lipids with respect to that of oil within barley seeds

This is an important consideration, since sterols, Ts, and T3s were all expressed with respect to oil yield. It also relates to production efficiency if one wants to produce a fraction concentrated

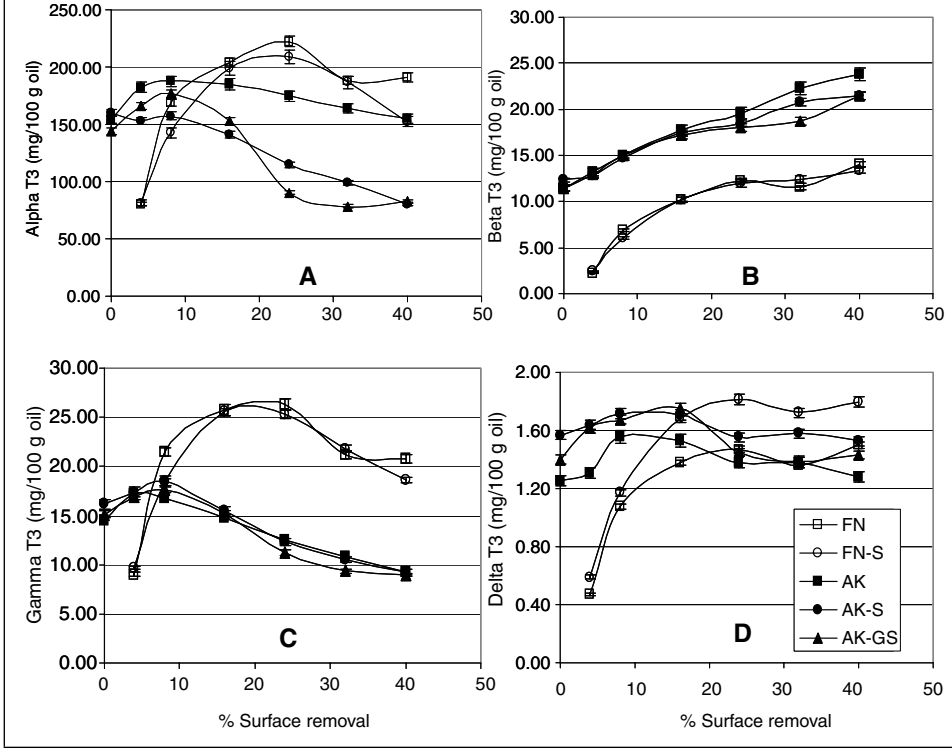


Figure 3—The yield and distribution of α (A), β (B), γ (C), and δ (D) tocotrienol isomers in abraded barley fines and kernels, and the effect of accelerated storage. FN = fines; FN-S = stored fines; AK = abraded kernels; AK-S = stored abraded kernels; AK-GS = ground and then stored abraded kernels.

in sterols, Ts, or T3s from barley seeds. By comparison of Figure 1B and 2 with Figure 1A, respectively, it is obvious that as the level of surface removal progressed, oil yield diminished, and the levels of phytosterol and all T isomers diminished also. Furthermore, T isomers decreased at a faster rate than oil. In contrast, comparison between Figure 3 and 1A shows that oil and T3 isomers had an opposite changing pattern; as the level of surface removal progressed, T3s showed an overall increase trend but the oil yield decreased.

Based on the results of this study, if one wants to concentrate sterols and Ts for production of functional lipids from hulless barley, it is more productive to focus on collecting fines from the initial 16% surface removal. If one wants to produce T3s, it is more productive to collect the tissues of aleurone and endosperm. Furthermore, if one wants to have a barley fraction enriched with all 3 types of functional lipids, sterols, Ts, and T3s, the aleurone layer is the most promising portion of the kernel on which to focus.

Confirmation of findings through the 2nd experiment of natural storage at ambient conditions

The previous discussion is based on results made with the 1st experiment using accelerated storage conditions. To confirm findings on yields and distributions of oil and functional lipids in barley seeds and effects of storage on them, we conducted a 2nd experiment where the samples were subjected to natural storage conditions (approximately 25 °C, indoors without moisture control) for 6 mo, using a different line of hulless barley. Data from this 2nd experiment (Table 2) confirmed all of the trends described in the 1st experiment. One major exception was for the free phytosterols, which did not show a consistent decreasing trend as a result of storage. Also, there were differences in absolute values when comparing fraction by fraction. In general, the amounts of all the measured attributes in the 2nd experiment were lower than those found in the 1st experiment. This is particularly true for the yield of total free sterols. The initial levels of free phytosterols were 3 to 4 g/100 g oil in the 1st experiment, but only about 1 g/100 g oil in the 2nd experiment. The differences in sterol values and changing trends with storage observed between the 2 storage experiments can be attributed partially to the effects of genotype and storage conditions since the 2 experiments used different barley lines and different storage conditions. Another possible explanation is that the 2 seed samples were grown at the different locations and harvested at different times.

Since the 2 experiments gave similar findings (except for free sterols) but used 2 different barley lines and 2 different storage conditions (an accelerated storage condition with a short duration, and a natural storage condition with a long duration), the observations and conclusions on concentrations in functional lipids in abraded fractions of hulless barley and effect of storage were more striking.

Further discussion

There is controversy with regard to relative concentrations of T, T3, and their isomers in barley tissues. Peterson (1994) reported that barley hulls and endosperm had substantial tocol concentrations, especially T3s, whereas the germ contained a high concentration of α-T and β-T3. Barnes and Taylor (1981) reported that the barley germ fraction had a very high concentration of α-T but contained no T3s. Because these studies used limited fractions for measurement, they did not provide a whole picture of tocols and their isomers across the barley kernel. Our study showed that barley germ contained the highest amounts of all T isomers but the lowest concentrations of all T3 isomers, whereas barley endosperm tissue contained almost no Ts and relatively high amount of T3s. In addition, the barley aleurone layer contained the highest amounts of T3s and relatively high amounts of Ts. Therefore, although the levels of total tocols are highest in the outer layers of barley, its inner core region also has a substantial amount of T3s.

Wang and others (1993) examined the concentrations of Ts and T3s in milling and pearling fractions of hulless barley. They found

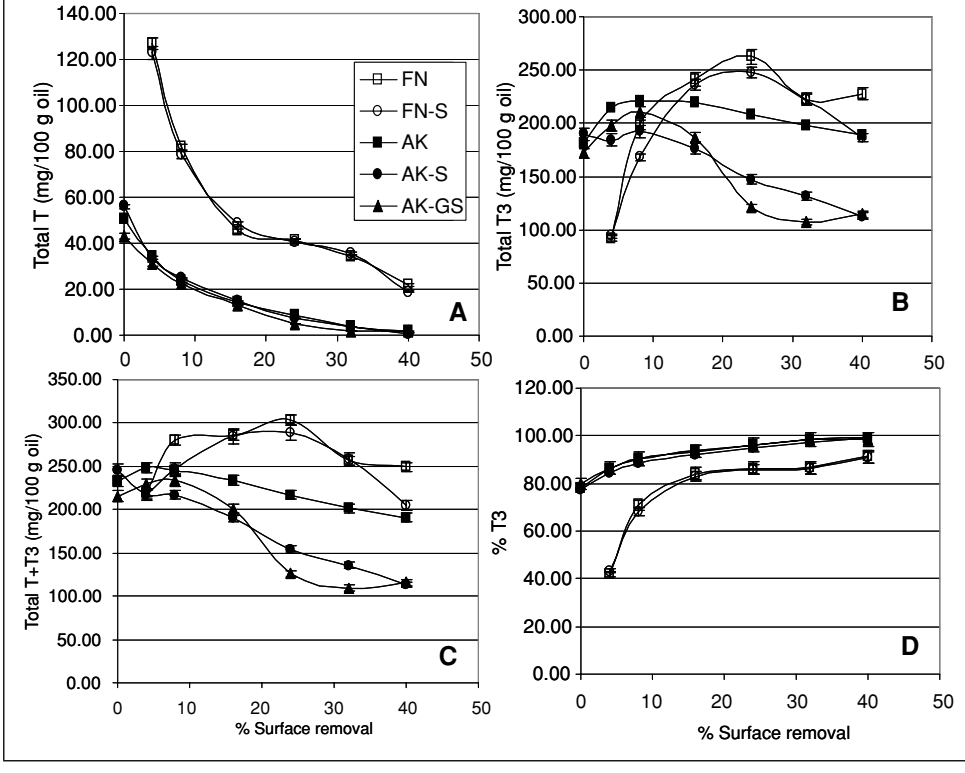


Figure 4—The yield and distribution of total tocopherol (A), total tocotrienol (B), sum of total tocopherol and tocotrienol (C), and percentage of tocotrienol (D) in abraded barley fines and kernels, and the effect of accelerated storage. FN = fines; FN-S = stored fines; AK = abraded kernels; AK-S = stored abraded kernels; AK-GS = ground and then stored abraded kernels.

Table 2—Oil yield and functional lipid components in abraded barley fines and kernels and effect of subsequent storage.[a]

Abrading percentage of removal	Storage (after abrasion)	Oil* percentage	Sterol* g/100 g oil	(mg/100 g Oil)											%T3
				αT	βT	γT*	δT*	αT3	βT3	γT3	δT3	Total T	Total T3	Total tocol	
Fines															
4	No	10.0 ± 0.3	0.71 ± 0.11	69.3 ± 1.9	3.0 ± 0.1	13.3 ± 0.3	2.0 ± 0.0	73.9 ± 1.9	1.4 ± 0.2	7.4 ± 0.3	0.4 ± 0.0	87.6	83.1	170.7	48.7
	Yes	10.4 ± 0.2	0.66 ± 0.09	65.4 ± 0.5	3.4 ± 0.1	13.8 ± 0.4	2.3 ± 0.1	61.4 ± 1.3	1.3 ± 0.0	7.2 ± 0.1	0.7 ± 0.0	84.9	70.7	155.5	45.4
8	No	9.5 ± 0.1	0.55 ± 0.18	31.7 ± 0.8	1.6 ± 0.1	5.6 ± 0.1	0.9 ± 0.0	151.9 ± 2.9	3.1 ± 0.0	16.5 ± 0.4	1.0 ± 0.1	39.9	172.5	212.4	81.2
	Yes	9.2 ± 0.1	0.54 ± 0.07	29.8 ± 0.4	1.9 ± 0.1	6.0 ± 0.3	1.1 ± 0.0	138.3 ± 1.3	3.4 ± 0.2	16.1 ± 0.2	1.1 ± 0.0	38.7	158.9	197.7	80.4
16	No	8.6 ± 0.1	0.48 ± 0.16	17.8 ± 0.3	1.1 ± 0.0	2.4 ± 0.1	0.6 ± 0.0	181.0 ± 1.9	4.1 ± 0.1	19.2 ± 0.3	1.2 ± 0.0	21.9	205.4	227.3	90.4
	Yes	8.3 ± 0.2	0.48 ± 0.05	15.3 ± 0.7	1.1 ± 0.1	2.4 ± 0.1	0.6 ± 0.0	158.7 ± 4.4	3.6 ± 0.1	17.2 ± 0.4	1.2 ± 0.1	19.3	180.6	199.9	90.4
24	No	5.1 ± 0.1	0.46 ± 0.09	14.8 ± 0.5	1.1 ± 0.0	1.7 ± 0.0	0.4 ± 0.2	150.0 ± 5.8	4.0 ± 0.1	16.6 ± 0.7	1.1 ± 0.1	18	171.8	189.8	90.5
	Yes	5.0 ± 0.1	0.46 ± 0.08	10.9 ± 0.4	1.1 ± 0.2	1.6 ± 0.2	0.6 ± 0.0	115.0 ± 1.1	3.7 ± 0.1	15.8 ± 0.3	1.1 ± 0.2	14.2	135.6	149.7	90.5
32	No	2.6 ± 0.1	0 ± 0	8.2 ± 0.6	1.1 ± 0.0	1.2 ± 0.1	0.4 ± 0.1	99.4 ± 3.9	4.0 ± 0.2	14.3 ± 0.7	1.3 ± 0.1	10.8	119.1	129.9	91.7
	Yes	2.5 ± 0.2	0 ± 0	3.8 ± 0.5	0.5 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	43.4 ± 4.1	3.8 ± 0.6	14.5 ± 1.4	0.7 ± 0.2	4.9	62.3	67.2	92.7
Kernels															
0	No	1.3 ± 0.1	1.01 ± 0.21	34.3 ± 1.4	2.1 ± 0.1	6.5 ± 0.2	1.3 ± 0.1	140.5 ± 5.9	5.0 ± 0.4	13.4 ± 0.7	1.0 ± 0.1	44.2	159.8	204	78.3
	Yes	1.4 ± 0.1	1.05 ± 0.13	33.6 ± 1.4	2.0 ± 0.2	6.0 ± 0.2	1.2 ± .01	129.0 ± 7.4	4.5 ± 0.3	12.1 ± 0.9	0.9 ± 0.1	42.8	146.5	189.3	77.4
4	No	1.6 ± 0.0	0.83 ± 0.24	14.1 ± 0.3	1.2 ± 0.0	2.5 ± 0.1	0.4 ± 0.1	134.4 ± 2.9	4.8 ± 0.1	13.3 ± 0.1	1.1 ± 0.0	18.2	153.5	171.6	89.4
	Yes	1.8 ± 0.2	0.44 ± 0.08	16.3 ± 2.0	1.1 ± 0.0	2.5 ± 0.2	0.6 ± 0.2	116.8 ± 9.7	4.0 ± 0.2	13.2 ± 1.0	0.9 ± 0.1	20.6	134.9	155.5	86.7
8	No	1.4 ± 0.1	0.80 ± 0.19	9.8 ± 0.1	1.0 ± 0.0	1.6 ± 0.1	0.3 ± 0.1	138.3 ± 3.8	5.5 ± 0.2	13.7 ± 0.4	1.2 ± 0.1	12.7	158.6	171.4	92.6
	Yes	1.4 ± 0.1	0.70 ± 0.32	9.9 ± 0.4	0.7 ± 0.0	1.5 ± 0.1	0.5 ± 0.0	112.0 ± 2.9	4.4 ± 0.1	13.4 ± 0.3	1.0 ± 0.0	12.5	130.8	143.4	91.3
16	No	1.0 ± 0.1	0.70 ± 0.09	6.1 ± 0.3	0.8 ± 0.0	1.0 ± 0.1	0.2 ± 0.0	123.9 ± 6.1	6.2 ± 0.3	11.2 ± 1.2	1.1 ± 0.3	8.1	142.4	150.5	94.6
	Yes	1.0 ± 0.0	0.73 ± 0.25	4.9 ± 0.4	0.8 ± 0.1	0.8 ± 0.1	0.1 ± 0.0	76.8 ± 4.3	5.3 ± 0.2	11.2 ± 0.2	1.1 ± 0.1	6.6	94.4	101	93.5
24	No	0.7 ± 0.1	0.79 ± 0.05	2.6 ± 0.5	0.4 ± 0.1	0.4 ± 0.1	0 ± 0	108.5 ± 15.4	8.1 ± 1.2	9.7 ± 1.2	0.5 ± 0.1	3.5	126.8	130.3	97.3
	Yes	0.7 ± 0.0	1.03 ± 0.17	1.4 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0 ± 0	40.8 ± 2.0	5.6 ± 0.4	8.1 ± 0.4	0.2 ± 0.0	1.8	54.7	56.5	96.8
32	No	0.4 ± 0.0	1.26 ± 0.11	0.4 ± 0.0	0 ± 0	0 ± 0	0 ± 0	106.9 ± 10.9	10.7 ± 1.2	9.3 ± 1.3	0.4 ± 0.0	0.4	127.4	127.9	99.7
	Yes	0.7 ± 0.1	0.95 ± 0.13	0.3 ± 0.0	0 ± 0	0 ± 0	0 ± 0	23.2 ± 1.5	5.3 ± 0.3	5.4 ± 0.1	0.2 ± 0.0	0.3	34	34.3	99.1

[a]Samples were stored in sealed plastic bags at ambient temperature (approximately 25 °C) and ambient relative humidity (a temperature controlled seed laboratory) for 6 mo.
The data are means ± SD. Storage had a significant effect ($P < 0.05$) on all attributes of abraded fractions except for those with * mark.

that pearling was more effective than milling as a means of concentrating total tocols and oil in barley flour. A pearling fines fraction consisting of 20% of the original kernel weight had the highest concentrations of α-T3, α-T, total tocols, and oil, 2.7, 4.4, 2.9, and 2.9 times greater, respectively, than those of the whole grain. Our current study confirmed that the seed surface layers contained most of the oil and oil-soluble components. We also demonstrated that concentrations of T, T3, and their isomers varied with tissues and thus the relative percentage of each component changed in fractions as the level of surface layer removal increased.

There are a very limited number of studies on the stability of tocols in barley and barley products (Hakkarainen and others 1983; Tyopponen and Hakkarainen 1985), but studies with other cereal species also offer some insights on the subject (Piironen and others 1988; Peterson 1995; Park and others 2004). In barley meal (Tyopponen and Hakkarainen 1985), wheat flour and rye meal (Piironen and others 1988), tocols degraded with time, but compared with barley flour, tocols in intact barley grain degraded at a slower rate (Hakkarainen and others 1983). In another report (Peterson 1995) tocols were found to be stable for 7 mo in all oat products in jars stored at a frozen temperature, but they were degraded when store at a room temperature in all processed products except for undried groats.

In the same report, Peterson (1995) observed that tocols degraded faster in envelopes than in jars during room temperature storage, and suggested that air (oxygen) might be involved in the degradation process. He also found that α-T and α-T3 degraded faster than the other homologues during room temperature storage in envelopes and suggested that there was differential stability among isomers. Park and others (2004) studied the comparative stability of tocols in rice bran oil at high temperatures and high oxygen concentrations and found that αT3 degraded more rapidly and more extensively than the other isomers. A differential degradation of homologues was also noted by Piironen and others (1988), who reported that in wheat flour and rye whole meal, α-T and α-T3 were degraded faster than β-T and β-T3. In contrast, Hakkarainen and others (1983) found that the isomer composition of tocols in barley seeds with a normal moisture level remained stable during 11-mo storage in the conventional silo even though the yield of total tocols had an average monthly loss of approximately 1%. Similarly, Tyopponen and Hakkarainen (1985) found that the composition of tocol homologues was unchanged when milled barley was stored at an ambient temperature for 8 wk or heated at temperatures up to 90 °C for 48 h.

Conclusions

The present study investigated the effect of storage on multiple aspects: type of storage (accelerated compared with natural), intact kernels, abraded fractions with different surface removal levels, total tocols, total Ts, total T3s, and homologues of Ts and T3s. The results show that there was differential degradation not only among homologues but also between Ts and T3s. There was also an effect of tissue-specific interaction. There is an agreement between this study and Park and others (2004) that α-T3 degraded more rapidly and severely than others during storage. Furthermore, the present study showed that the direction of change as a result of storage varied with T3 homologues (for example, δ-T3 increased instead of decreased), and that changes of T3s during storage were more pronounced in fractions containing more inner core tissues. As a result, the isomer composition of T3 changed during storage. Unlike T3s, Ts were relatively stable during storage and thus homologue composition of Ts remained stable also. These results provide practical information to those who wish to produce a barley fraction enriched with a particular functional lipid and maintain stability of their products.

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